ANTIBACTERIAL ACTIVITY OF ETHANOLIC AND METHANOLIC SEED EXTRACT OF FIVE CASSIA SPECIES AGAINST SOME PATHOGENIC BACTERIA

Najila Saleh Mahdi Abdelsaheb Alasady[1], Attyaf Jameel Thamir[2]

[1,2]University of Kufa, Faculty of Science, Biology Department, najlaas.abdulsahib@uokufa.edu.iq, atyaf.altameemi@uokufa.edu.iq

Abstract
Current investigation was accomplished at biology department/faculty of science/kufa university during 2023-2024. Seeds organic solvent extract was prepared using methanol and ethanol by Soxhlet) at three concentration (100mg/ml/,200mg/ml and 400 mg/ml) for five Cassia species (1-C. fistula, 2-C.bakeriana, 3-C.gluaca , 4-C.siamea 5-C.alata) . examined bacterial strain were resistance to many antibiotics. slight variation observed between cassia species and between methanol and ethanol extract, high concentration produced highest bacterial growth inhibition. E.coli gave highest growth inhibition zone compared to S. aureus and K. pneumoniae . Both methanolic and ethanolic extract of examined Cassia species possess antibacterial activity against examined bacterial strains especially with increasing concentration compared to control (DMSO) treatment with exception of C.fistula methanolic extract at concentration of 100mg/ml against S. aureus and C.alata ethanolic extract at concentration of 100mg/ml against K. pneumonia ,both failed to give any antibacterial activity. C.siamea methanolic extract at concentration of 400mg/ml gave highest antibacterial activity against E.coli.
Keywords: Cassia, antibacterial

Introduction

Since the introduction of the antibiotics, they have been known as one of the most important tools against bacterial infections. Although, in the past years due to the widespread and in some cases incorrect use of antibiotics we can see dramatic increase in microbial resistance against antimicrobial agents that is a critical reason for finding new drugs with les resistance and side effects (Bhalodia and Shukla, 2011).

The medicinal plants have been used from ancient times especially in Asia, and have been used for the treatment of specific illness (Seyyednejad et al.,2014), the raise of antibiotic resistant pathogens is a growing concern all over the world and WHO assign it as an emerging public health problem (Soltan et al.,2007)).

Bacteria such as *Escherichia coli* and *Klebsiella spp*., the main agents of UTI (urinary tract infection) (Golamreza and Ali.,2011).

Herbal antimicrobials have contributed to the control of various diseases caused by icroorganisms (Anand et al.,2019). The World Health Organization (WHO) encourages, recommends, and promotes the inclusion of herbal medicines in national health care systems (WHO.,2019) This is due to the fact that they are easily available at low rates and are thought to be safer than modern synthetic medications (Alam and Mishra,2017)

It has been estimated that 80% of the world's population uses traditional plant medicines to meet their primary health care needs (WHO, 2017). *Cassia L.* is a big flowering plant genus (Fabaceae) with three subgenera including *Cassia L.,* Senna Mill., and Chamaecrista Moench. Recently, each of these subgenera has been upgraded into genera using molecular characters, notably DNA markers (Zibaee et al.,2023).

Some of them are used in traditional folk medicines as a laxative, purgative, antimalarial, ulcer healing, antidiabetic, hepatoprotective, nephroprotective, antitumor and also used in the treatment of skin infection and periodic fever throughout the tropical and subtropical region (Hafez et al.,2019)

Genus *Cassia* revealed the isolation and separation of different following classes of compounds: A number of authors isolated and identified several compounds from different Cassia species such as anthraquinones, anthracenes, polyphenols, fatty acids, sterols, polysaccharides and some other miscellaneous compounds from different *Cassia* species (Caro et al.,2012)

Material and methods

Seeds of five Cassia species including (1-*C. fistula,2-C.bakeriana, 3-C.gluaca , 4-C.siamea 5-C.alata*) as in Figure (1), leaf and flowers as in figure (2)

Figure (1) Cassia species seeds including (1-C.fistula, 2-C.bakeriana , 3-C.gluaca, 4-C.siamea 5-C.alata)

Figure (2) Cassia species leaf and flowers including (C.fistula, C.bakeriana, C.gluaca)

Plant extract preparation

Seeds of five Cassia species including (1-C.fistula, 2-C.bakeriana, 3-C.gluaca, 4-C.siamea 5-C.alata) were grineded individually using electrical grinder . Using 30g of each crushed plant material sample and loaded into the extraction thimble, 500 ml of (absolute ethanol and absolute methanol) separately was added to a round bottom flask, and heated at 40C° for 24 hours. Recovery of stock plant extract was done by heating at 40 C° using oven till drying. This extract consider as stock solution and used later to prepare different concentration (Al-Mohammad.,2010).

Micro organism growth inhibition

Bacterial (Escherichia coli, Staphylococcus aureus, klebsiella pneumoniae) samples were provided and identified from Al-Ameen labrotary /Holy Shrine.

Antibacterial activity

Preparation of bacterial suspension

The bacterial suspension was prepared by culturing bacteria on nutrient agar and incubation for 24 h at 37 C°, after 3-4 weeks, colony were taken to test tube contenting on nutrient broth and incubation for 4-5 h in 37 C° , later comparing with McFarland tube to obtained suitable turbidity . The turbidity produced by growth culture was calibrated with sterilized broth to achieve an optical density comparable to the 0.5 McFarland requirements (1.5 X 108 cells/ml the equivalent).

Antibiotic sensetivity test

Testing sensitivity of bacteria to some antibiotic was done using disc diffusion method by culturing each genus of bacteria on Muller Hinton agar ,then, spreading 0.1ml of bacterial suspension (which comparison with McFarland tube 0.5) on agar surface and put the antibiotic disc in each plate after then incubator all plate at 37 C for 24 h ,later measuring of inhibition zone(Josiphine et al.,2006)

Plant extract activity test

Plant extract examined at three concentrations (100mg /ml, 200 mg /ml, 400mg / ml) by dilution using DMSO using method of agar diffusion by well was used in susceptibility test for plant extract by making equal well in Muller Hinton with diameter of 6mm by cork borer and adding of 0.1 ml from each extract, before this step, spreading of o.1 ml from bacterial suspension on surface of media ,after then incubation the plate over night in 37 C° and then measuring the diameter of inhibition zone to detect the activity of test plant extract on growth of bacteria using ruller (Egorove .,1985)

Results

Antibiotic sensetivity

Results shown in table (1) indicates that examined bacterial strains are resistant to many antibiotics, thus it was ideal to examine plant extract against them.

Table (1) Antibiotic sensitivity test for bacteria used in current study

Antibiotic	Bacterial response to antibiotic		
	S. aureus, (gram posotive)	*K. peunemoniae (gram negative)*	*E.coli (gram negative)*
CRO	R	R	R
CAZ	R	R	R
CTX	R	R	S
CFM	R	R	R
F	S	S	R
CN	R	S	S
AK	R	S	S
TE	R	R	R
TMP	R	S	S
APX	R	R	R
ATM		R	S
AX	R	R	R
NA	R	R	S
CIP	R	S	S
NOR	R	S	S
OFX	R	S	S
IPM		S	S
MEM		S	S
CO		S	R
PRL		R	R
PB		S	
AZM	R		
E	R		

Microorganisms can be either intrinsically resistant to an antibiotic or develope resistance following exposure to that antibiotic (acquired resistance), resistance can develop as a result of mutation or direct transfer of genes encoding a resistance mechanism (Livermore.,2004)

Genetic material, including antibiotic resistance genes, can spread very effectively between bacteria, even those of unrelated species, antibiotic resistance can be considered to be an inevitable consequence of antibiotic use, injudicious use of antibiotics is a major factor facilitating the emergence of resistance worldwide (Sabtu et al.,2016).

Table (2) Interaction among cassia species, extract type, concentation and bacterial strain in antibacterial activity (measured in mm)

Cassia species	Concentrations mg/ml	Bacterial strain					
		Methanolic extract			Ethanolic extract		
		S. aureus,	*E.coli*	*K. pneumoniae*	*S. aureus,*	*E.coli*	*K. pneumoniae*
	Control	0	0	0	0	0	0
1-*C.fistula*	400	20	26	23	22	25	24
	200	14	19	16	17	21	19
	100	0	14	11	12	16	15
2-*C.bakeriana*	400	20	25	24	22	28	24
	200	13	18	17	18	21	19
	100	11	13	14	13	17	16
3-*C.gluaca*	400	21	27	24	21	22	23
	200	14	18	17	16	17	18
	100	11	14	13	12	14	13
4-*C.siamea*	400	18	30	28	22	23	20
	200	17	22	19	17	18	15
	100	12	15	14	13	14	12
5-*C.alata*	400	22	26	25	25	24	22
	200	18	20	17	18	19	17
	100	13	15	14	12	13	0
L.S.D. 0.05 interaction		0.218					

Results in table (2) show that both methanolic and ethanolic extract of examined Cassia species possess antibacterial activity against examined bacterial strains especially with increasing concentration compared to control (DMSO) treatment with exception of C.fistula methanolic extract at concentration of 100mg/ml against S. aureus and C.alata ethanolic extract at concentration of 100mg/ml against K. pneumonia ,both failed to give any antibacterial activity . C.siamea methanolic extract at concentration of 400mg/ml gave highest antibacterial activity against E.coli (30mm)

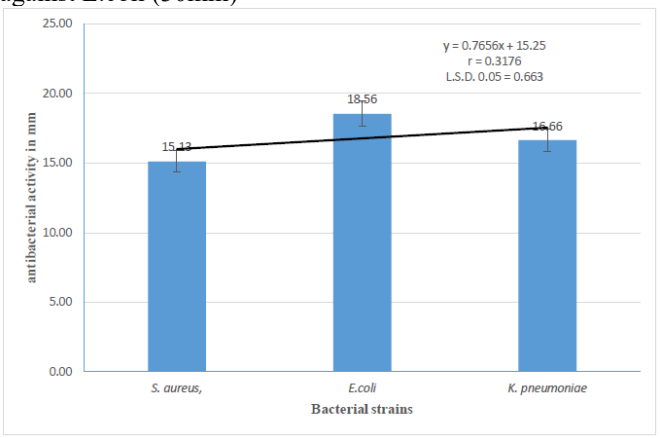

Figure (3) effect of bacterial strain (S. aureus, E.coli and K. pneumonia) in antibacterial activity (measured in mm)

Results in figure (3) indicate that E.coli gave highest growth inhibition zone (18.56 mm) compared to S. aureus (15.13mm) and K. pneumoniae (16.66mm)

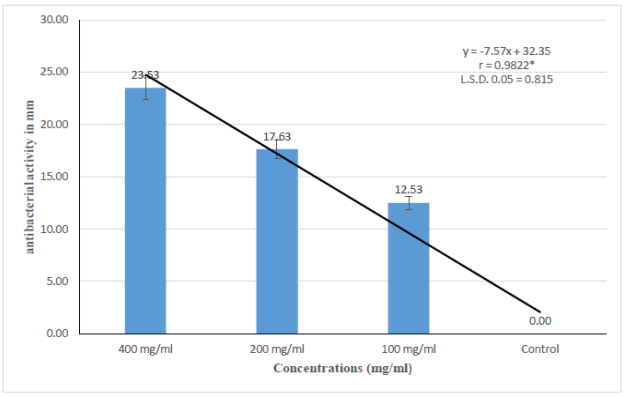

Figure (4) effect of plant extract concentration (100mg/ml, 200mg/ml, 400mg/ml) in addition to control treatment (DMSO) on antibacterial activity (measured in mm)

Results in figure (4) showed that concentration 400mg/ml gave highest growth inhibition zone (23.53mm) compared to concentration 200mg/ml (17.63 mm) and concentration 100mg/ml which gave (12.53mm)

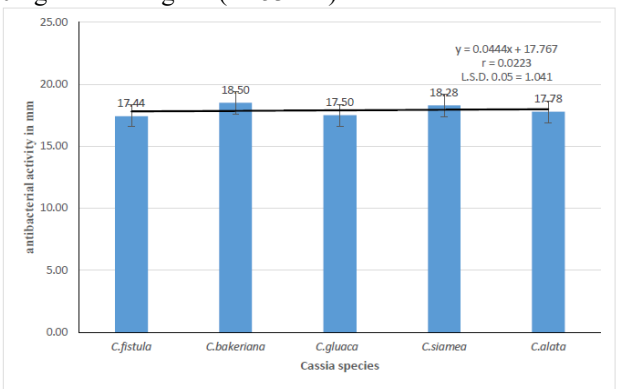

Figure (5) effect of cassia species (-C.fistula , 2-C.bakeriana , 3-C.gluaca, 4-C.siamea 5-C.alata) on antibacterial activity (measured in mm)

Figure (5) indicate that slight variation was observed among Cassia in their antibact C.erial values, C.fistula gave lowest value but it does not significantly differ with C.gluaca and C.alata while both C.bakeriana and C.siamea gave highest value but they does not differ between them

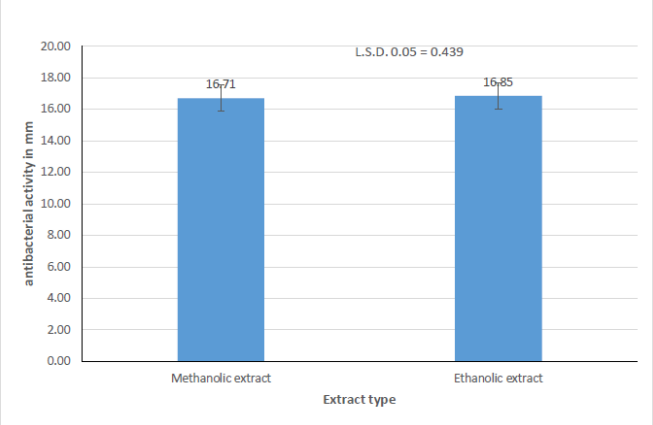

Figure (6) effect of plant extract type (methanol or ethanol) on antibacterial activity (measured in mm)

Figure (6) showed that there was no significant differences between methanol and ethanol solvent in their effect on antibacterial activity

Figures 7-16 show how plant extract with increase concentration increase in their bacterial growth inhibition by increase growth inhibition zone.

Figure (7) Ethanolic seed extract of C.fistula against S. aureus, E.coli and K. pneumoniae at three concentration 1-400mg/ml, 2-200mg/ml ,3- 100mg/ml in addition to control treatment (c) using (DMSO)

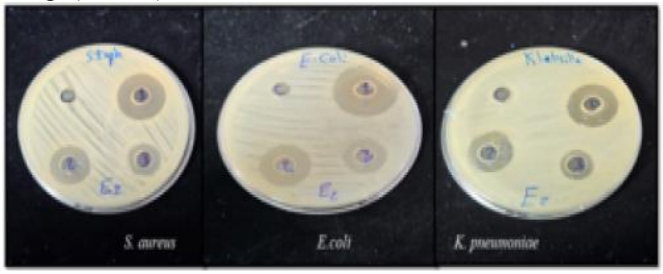

Figure (8) Ethanolic seed extract of C.bakeriana against S. aureus, E.coli and K. pneumoniae at three concentration 1-400mg/ml, 2-200mg/ml ,3- 100mg/ml in addition to control treatment (c) using (DMSO)

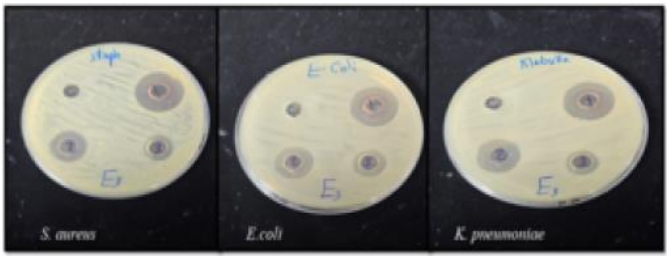

Figure (9) Ethanolic seed extract of C.gluaca against S. aureus, E.coli and K. pneumoniae at three concentration 1-400mg/ml, 2-200mg/ml ,3- 100mg/ml in addition to control treatment (c) using (DMSO)

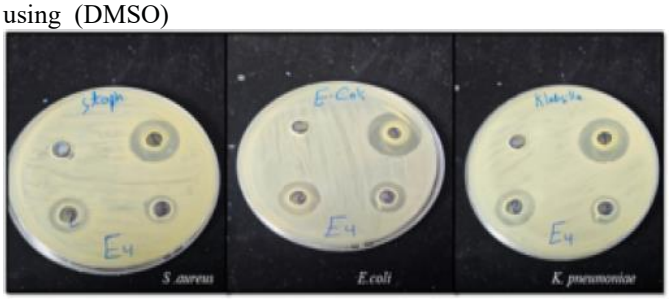

Figure (10) Ethanolic seed extract of C.siamea against S. aureus, E.coli and K. pneumoniae at three concentration 1-400mg/ml, 2-200mg/ml, 3- 100mg/ml in addition to control treatment (c) using (DMSO)

Figure (11) Ethanolic seed extract of C.alata against S. aureus, E.coli and K. pneumoniae at three concentration 1-400mg/ml, 2-200mg/ml, 3- 100mg/ml in addition to control treatment (c) using (DMSO)

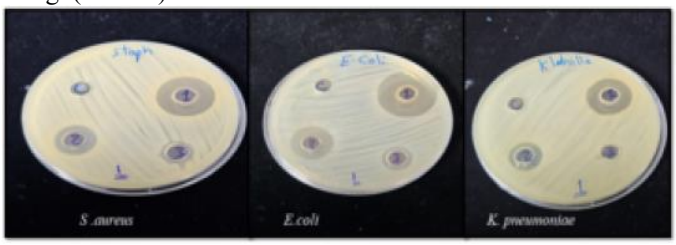

Figure (12) Methanolic seed extract of C.fistula against S. aureus, E.coli and K. pneumoniae at three concentration 1-400mg/ml, 2-200mg/ml, 3- 100mg/ml in addition to control treatment (c) using (DMSO)

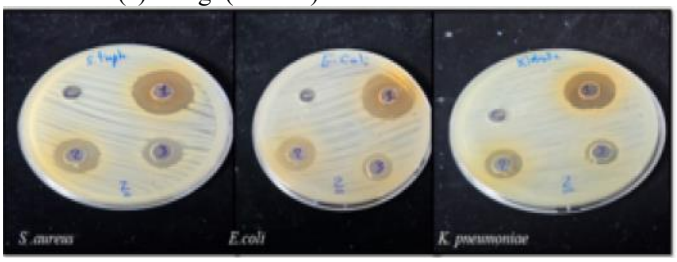

Figure (13) methanolic seed extract of C.bakeriana against S. aureus , E.coli and K. pneumoniae at three concentration 1-

400mg/ml, 2-200mg/ml ,3- 100mg/ml in addition to control treatment (c) using (DMSO)

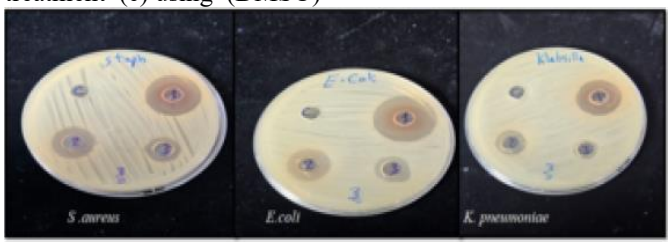

Figure (14) Methanolic seed extract of C.gluaca against S. aureus, E.coli and K. pneumoniae at three concentration 1-400mg/ml, 2-200mg/ml, 3- 100mg/ml in addition to control treatment (c) using (DMSO)

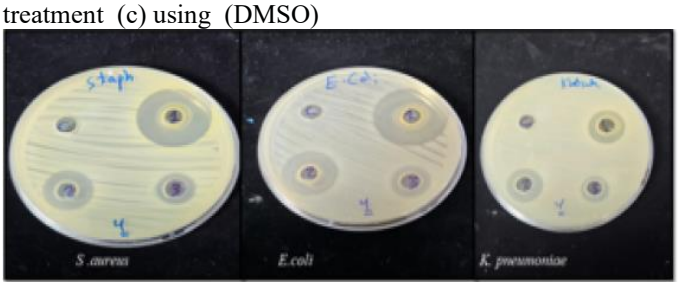

Figure (15) Methanolic seed extract of C.siamea against S. aureus, E.coli and K. pneumoniae at three concentration 1-400mg/ml, 2-200mg/ml, 3- 100mg/ml in addition to control treatment (c) using (DMSO)

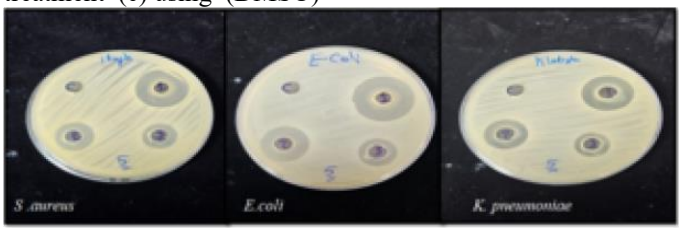

Figure (16) Methanolic seed extract of C.alata against S. aureus, E.coli and K. pneumoniae at three concentration 1-400mg/ml, 2-200mg/ml, 3- 100mg/ml in addition to control treatment (c) using (DMSO)

Discussion

Inhibitory concentrations of various plant extracts affect the virulence of both gram positive and gram negative bacteria (Thakur et al.,2016). Bacteria used efflux pumps as a mechanism to develop resistance to antibiotics so interfering with the functions of these efflux pumps would be an interesting therapy to control infectious diseases, besides inhibiting the function of efflux pumps, various plant extracts affect the virulence factors of bacteria with altering the quorum sensing (QS) gene expression (Holler et al.,2012).

In addition, various plant extracts have immunomodulatory effects. Since the plant extracts consist of complex mixture of phytochemical constituents, emergence of microbial resistance to the complex mixture of compounds may be much slower than those of antibiotics (Upadhyaya et al.,2013).

Different phyto-constituents have different degrees of solubility in different types of solvents depending on their polarity. In a traditional setting water is largely solvent used to prepare these concoctions (Elmahmood and Amey, 2007).

Methanol was reported AS an excellent solvent since phytochemicals like alkaloids, flavonoids, tannins and saponins can dissolve well in methanol(Kavimani et al.,2015).

Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defence mechanism against invasion by many microorganisms, insects and other herbivores. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell. Tannins bind to proline rich proteins and interfere with the protein synthesis.(Murugan et al.,2013).

The reason for the dissimilar sensitivity between gram positive and gram negative bacteria could be attributed to the morphological differences in the cell wall between these microorganisms. Gram negative bacteria have an outer phospholipid membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes (Gupta et al., 2009)

Conclusion

Both ethanol and methanol are an excellent solvent n plant phytochemical extraction. Cassia species possess antibacterial activity increased with increasing plant extract concentration.

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